MVRTAVLILLLVRFSEPKHKLEQLRNSGAAAEEEEYMPMEMNNSTNSSNNSLALTSPYKT
FEVVFIVLVAGSLSLVTIIGNILVMVSIKVNRHLQTVNNYFLFSLACADLIIGVFSMNLYTLYTV
IGYWPLGPVVCDLWLALDYVVSNASVMNLLIISFDRYFCVTKPLTYPVKRTTKMAGMMIAAAWVL
SFILWAPAILFWQFIVGVRTVEDGECYIQFFSNAAVTFGTAIAAFYLPVIIMTVLYWHISRASKS
RIKKDKKEPVANQDPVSPSLVQGRIVKPNNNNMPSSDDGLEHNKIRDPVTENCVQGEEKESSNDS
TSVSAVASNMRDDEITQDENTVSTSLGHSKDENSKQTCIRIGTKTPKSDSCTPTNTTVEVVGSSG
QNGDEKQNIVARKIVKMTKQPAKKKPPPSREKKVTRTILAILLAFIITWAPYNVMVLINTFCAPC
IPNTVWTIGYWLCYINSTINPACYALCNATFKKTFKHLLMCHYKNIGATR (SEQ ID NO:19)

FIG. 1

EXPRESS MAIL LABEL NO. EM300819915US
DATE OF DEPOSIT 12/19/01

MKFLVNVALVFMVVYISYIYADYKDDDDKMGQPGNGSAFLLAPNRSHAPDHDVTQQRDEV
WVVGMGIVMSLIVLAIVFGNVLVITAIAKFERLQTVTNYFITSLACADLVMGLAVVPFGAAHILM
KMWTFGNFWCEFWTSIDVLCVTASIETLCVIAVDRYFAITSPFKYQSLLTKNKARVIILMVWIVS
GLTSFLPIQMHWYRATHQEAINCYANETCCDFFTNQAYAIASSIVSFYVPLVIMVFVYSRVFQEA
KRQLQKIDKSEGRFHVQNLSQVEQDGRTGHGLRRSSKFCLKEHKALKTLGIIMGTFTLCWLPFFI
VNIVHVIQDNLIRKEVYILLNWIGYVNSGFNPLIYCRSPDFRIAFQELLCLRRSSLKAYGNGYSS
NGNTGEQSGYHVEQEKENKLLCEDLPGTEDFVGHQGTVPSDNIDSQGRNCSTNDSLLEEEEYMPM
E (SEQ ID NO:20)

FIG. 2

MKTIIALSYIFCLVFAMAILPAAETWIDGGGGVGADAVNLTASLAAGAATGAVETGWLQL
LDQAGNLSSSPSALGLPVRSPAPSQPWANLTNQFVQPSWRIALWSLAYGVVVAVAVLGNLIVIWI
ILAHKRMRTVTNYFLVNLAFSDASMAAFNTLVNFIYALHSEWYFGANYCRFQNFFPITAVFASIY
SMTAIAVDRYMAIIDPLKPRLSATATKIVIGSIWILAFLLAFPQCLYSKTKVMPGRTLCFVQWPE
GPKQHFTYHIIVIILVYCFPLLIMGITYTIVGITLWGGEIPGDTCDKYHEQLKAKRKVVKMMIIV
VMTFAICWLPYHIYFILTAIYQQLNRWKYIQQVYLASFWLAMSSTMYNPIIYCCLNKRFRAGFKR
AFRWCPFIKVSSYDELELKTTRFHPNRQSSMYTVTRMESMTVVFDPNDADTTRSSRKKRATPRDP
SFNGCSRRNSKSASATSSFISSPYTSVDEYSQPELAPEDPEDAAKHKLEQLRNSG (SEQ ID
NO:21)

FIG. 3

MKFLVNVALVFMVVYISYIYADYKDDDDKMNTSAPPAVSPNITVLAPGKGPWQVAFIGIT
TGLLSLATVTGNLLVLISFKVNTELKTVNNYFLLSLACADLIIGTFSMNLYTTYLLMGHWALGTL
ACDLWLALDYVASNASVMNLLLISFDRYFSVTRPLSYRAKRTPRRAALMIGLAWLVSFVLWAPAI
LFWQYLVGERTVLAGQCYIQFLSQPIITFGTAMAAFYLPVTVMCTLYWRIYRETENRARELAALQ
GSETPGKGGGSSSSSERSQPGAEGSPETPPGRCCRCCRAPRLLQAYSWKEEEEEDEGSMESLTSS
EGEEPGSEVVIKMPMVDPEAQAPTKQPPRSSPNTVKRPTKKGRDRAGKGQKPRGKEQLAKRKTFS
LVKEKKAARTLSAILLAFILTWTPYNIMVLVSTFCKDCVPETLWELGYWLCYVNSTINPMCYALC
NKAFRDTFRLLLLCRWDKRRWRKIPKRPGSVHRTPSRQCEEEEYMPME (SEQ ID NO:22)

FIG. 4

MKTIIALSYIFCLVFAMTLHSNSTTSPLFPNISSSWVHSPSEAGLPLGTVTQLGSYNISQ
ETGNFSSNDTSSDPLGGHTIWQVVFIAFLTGFLALVTIIGNILVIVAFKVNKQLKTVNNYFLLSL
ACADLIIGVISMNLFTTYIIMNRWALGNLACDLWLSIDYVASNASVMNLLVISFDRYFSITRPLT
YRAKRTTKRRGVMIGLAWVISFVLWAPAILFWQYFVGKRTVPPGECFIQFLSEPTITFGTAIAAF
YMPVTIMTILYWRIYKETEKRTKELAGLQASGTEAEAENFVHPTGSSRSCSSYELQQQGVKRSSR
RKYGRCHFWFTTKSWKPSAEQMDQDHSSSDSWNNNDAAASLENSASSDEEDIGSETRAIYSIVLK
LPGHSSILNSTKLPSSDNLQVSNEDLGTVDVERNAHKLQAQKSMGDGDNCQKDFTKLPIQLESAV
DTGKTSDTNSSADKTTATLPLSFKEATLAKRFALKTRSQITKRKRMSLIKEKKAAQTLSAILLAF
IITWTPYNIMVLVNTFCDSCIPKTYWNLGYWLCYINSTVNPVCYALCNKTFRTTFKTLLLCQCDK
RKRRKQQYQQRQSVIFHKRVPEQALQPELAPEDPEDAAHHHHHHH (SEQ ID NO:23)

FIG. 5

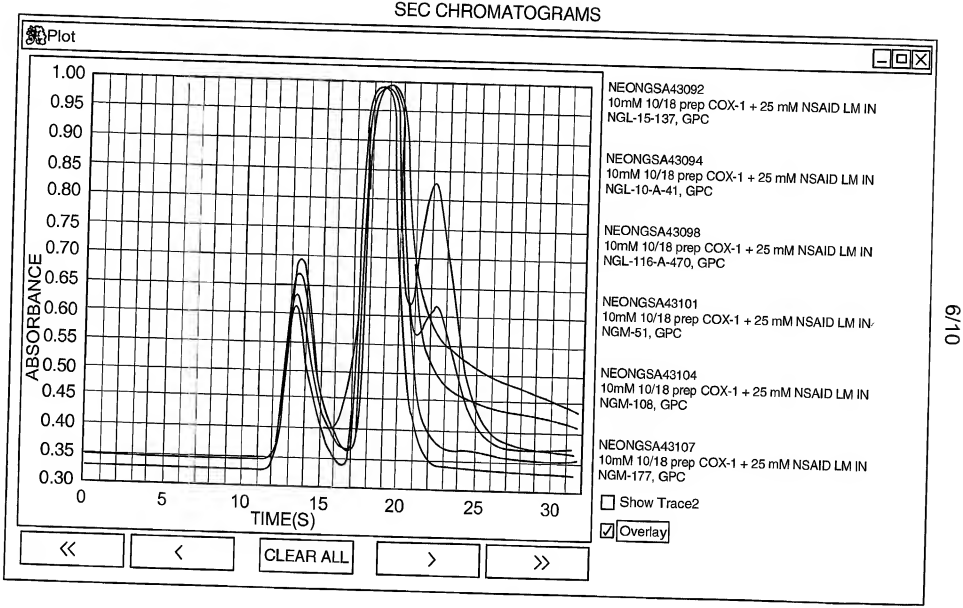
SEC CHROMATOGRAMS
Plot
1.00
0.95
0.90
0.85
0.80
0.75
0.70
0.65
0.60
0.55
0.50
0.45
0.40
0.35
0.30
ABSORBANCE
0
5
10
15
20
25
30
TIME(S)
NEONGSA43092
10mM 10/18 prep COX-1 + 25 mM NSAID LM IN
NGL-15-137, GPC
NEONGSA43094
10mM 10/18 prep COX-1 + 25 mM NSAID LM IN
NGL-10-A-41, GPC
NEONGSA43098
10mM 10/18 prep COX-1 + 25 mM NSAID LM IN
NGL-116-A-470, GPC
NEONGSA43101
10mM 10/18 prep COX-1 + 25 mM NSAID LM IN
NGM-51, GPC
NEONGSA43104
10mM 10/18 prep COX-1 + 25 mM NSAID LM IN
NGM-108, GPC
NEONGSA43107
10mM 10/18 prep COX-1 + 25 mM NSAID LM IN
NGM-177, GPC
Show Trace2
Overlay
CLEAR ALL

FIG. 6

COX-1 ALIS with Test Libraries

NSAID LIGAND MIX RECOVERY, COX-1 METHODS DEVELOPMENT 10-19-00

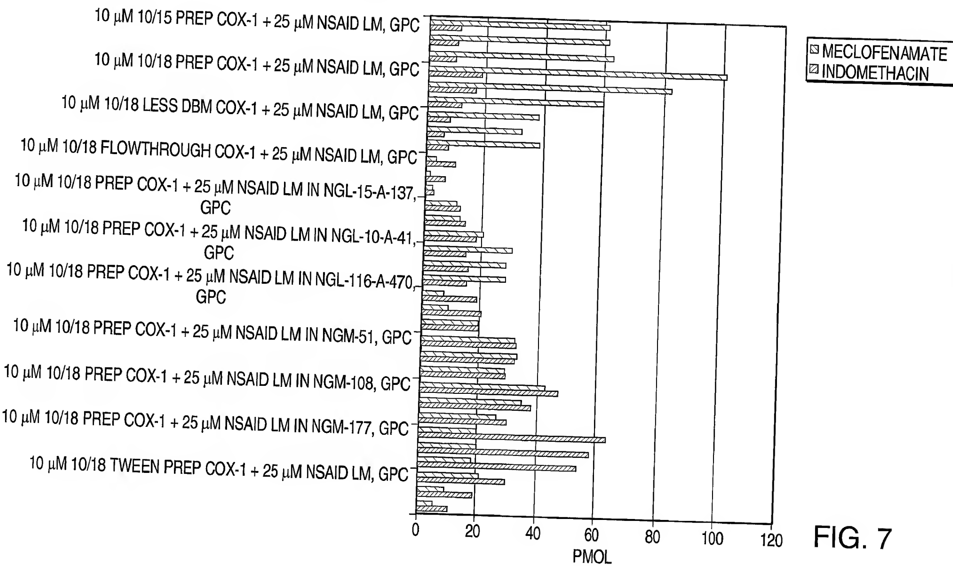

FIG. 7

Example COX-1 Ligand Identitied by ALIS

<u>NGL-177-A-1128-A-2</u>

$C_{31}H_{31}N_3O_5$

MW = 525.6

MASS = 525.23

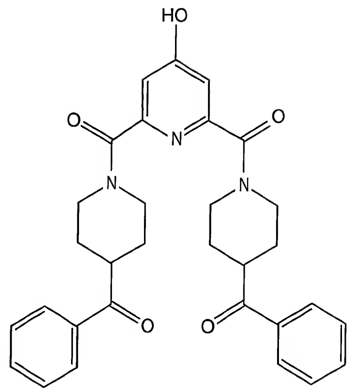

FIG. 8

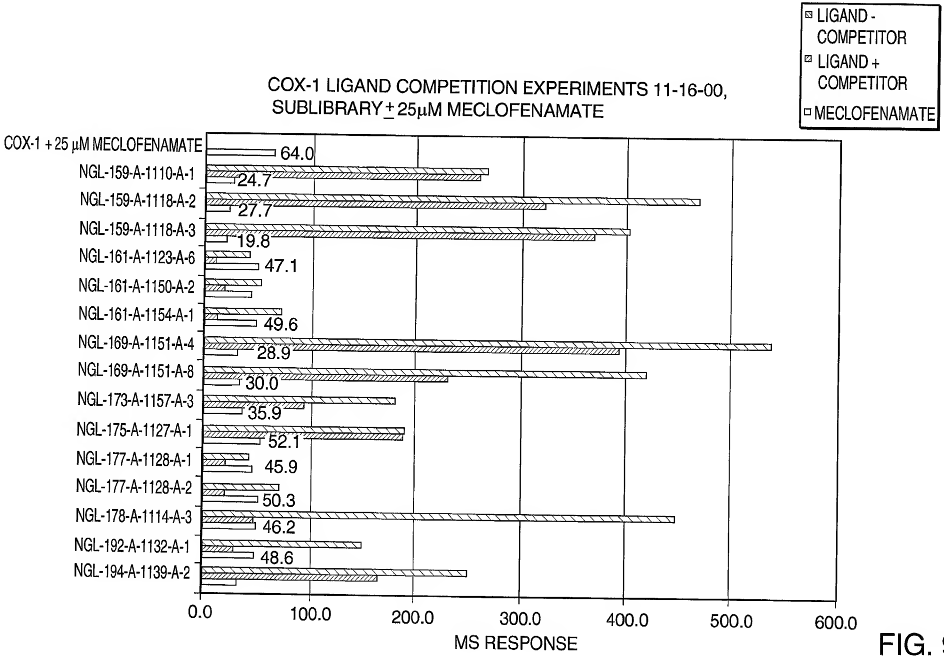
COX-1 LIGAND COMPETITION EXPERIMENTS 11-16-00,
SUBLIBRARY ± 25μM MECLOFENAMATE
LIGAND - COMPETITOR
LIGAND + COMPETITOR
MECLOFENAMATE
COX-1 + 25 μM MECLOFENAMATE
NGL-159-A-1110-A-1
NGL-159-A-1118-A-2
NGL-159-A-1118-A-3
NGL-161-A-1123-A-6
NGL-161-A-1150-A-2
NGL-161-A-1154-A-1
NGL-169-A-1151-A-4
NGL-169-A-1151-A-8
NGL-173-A-1157-A-3
NGL-175-A-1127-A-1
NGL-177-A-1128-A-1
NGL-177-A-1128-A-2
NGL-178-A-1114-A-3
NGL-192-A-1132-A-1
NGL-194-A-1139-A-2
64.0
24.7
27.7
19.8
47.1
49.6
28.9
30.0
35.9
52.1
45.9
50.3
46.2
48.6
0.0
100.0
200.0
300.0
400.0
500.0
600.0
MS RESPONSE

FIG. 9

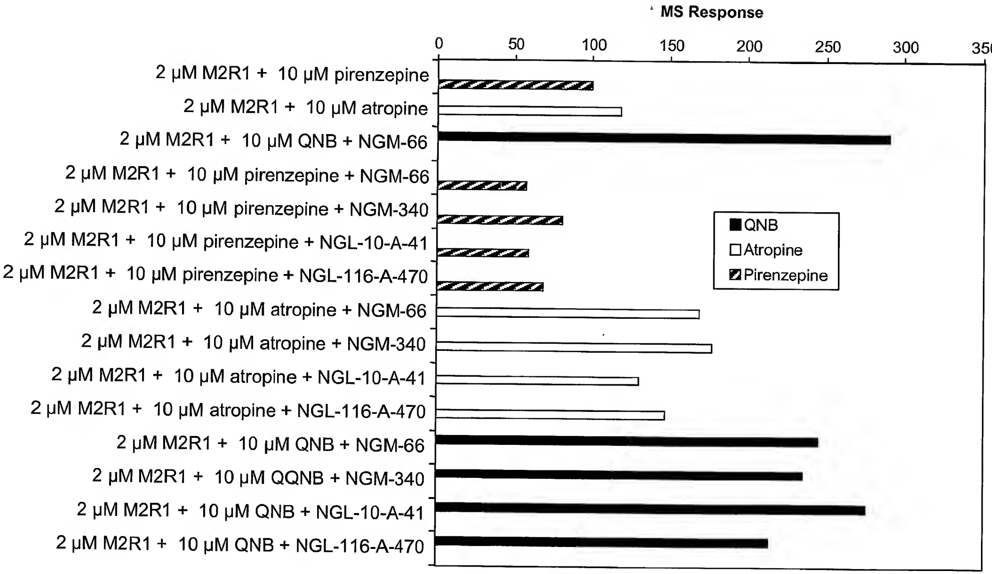
MS Response
0
50
100
150
200
250
300
350
QNB
Atropine
Pirenzepine
2 μM M2R1 + 10 μM pirenzepine
2 μM M2R1 + 10 μM atropine
2 μM M2R1 + 10 μM QNB + NGM-66
2 μM M2R1 + 10 μM pirenzepine + NGM-66
2 μM M2R1 + 10 μM pirenzepine + NGM-340
2 μM M2R1 + 10 μM pirenzepine + NGL-10-A-41
2 μM M2R1 + 10 μM pirenzepine + NGL-116-A-470
2 μM M2R1 + 10 μM atropine + NGM-66
2 μM M2R1 + 10 μM atropine + NGM-340
2 μM M2R1 + 10 μM atropine + NGL-10-A-41
2 μM M2R1 + 10 μM atropine + NGL-116-A-470
2 μM M2R1 + 10 μM QNB + NGM-66
2 μM M2R1 + 10 μM QQNB + NGM-340
2 μM M2R1 + 10 μM QNB + NGL-10-A-41
2 μM M2R1 + 10 μM QNB + NGL-116-A-470

FIG. 10